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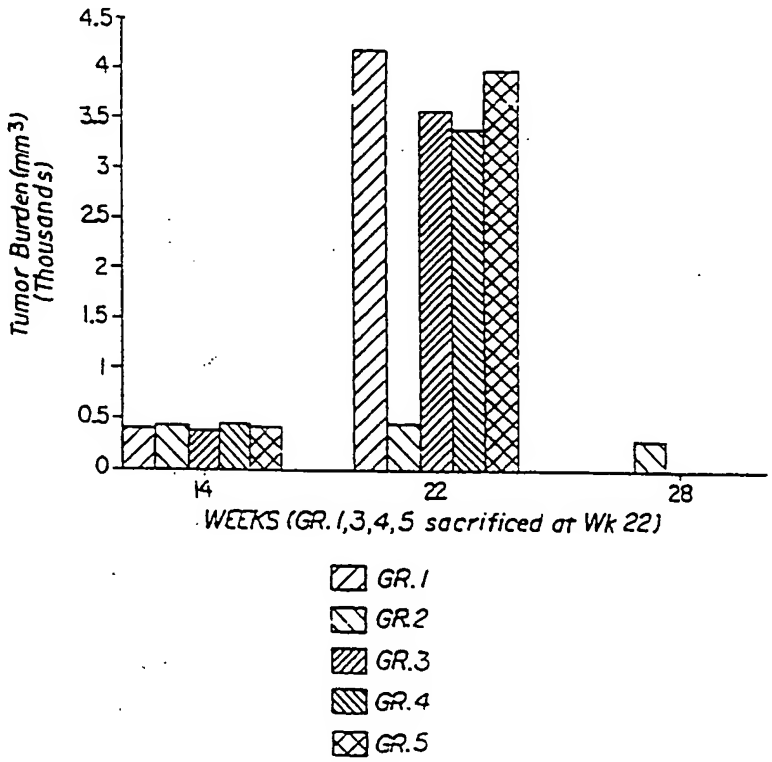
(54) Title: COMPOSITION AND METHOD FOR INDUCING TUMOR REGRESSION



(57) Abstract

Oral ingestion of a mixture of vitamin E and a carotenoid in a carrier induces regression of a tumor in a mammal.

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COMPOSITION AND METHOD FOR INDUCING TUMOR REGRESSION

This invention relates to a method and composition for inducing regression of tumors with a mixture of vitamin E and a carotenoid, and pertains more specifically to oral ingestion of a mixture comprising vitamin E and a carotenoid.

Vitamin E and ß-carotene has each been reported individually to be capable of inducing regression of epidermoid carcinomas in vivo in mammals when injected locally: Schwartz, et al. Biochem. Biophys. Res. Commun. Vol 136:1130-1135 (1986); Shklar et al. J.N.C.I. Vol 78:987-992 (1987); and, Schwartz et al. Nutr. Cancer. Vol 11:35-40 (1988).

It has now been found that a mixture of vitamin E and a carotenoid such as ß-carotene is remarkably effective in inducing regression of tumors such as epidermoid carcinomas in mammals when ingested orally by the mammal, whereas neither vitamin E nor ß-carotene when ingested individually is effective.

The drawing is a graphical representation of the results of practicing the present invention (Group 2), as well as the results obtained with controls and with administration of vitamin E and ß-carotene individually.

The term "vitamin E" as used in the present application is intended to include vitamin E from natural sources as well as pure alpha-tocopherol, alpha-tocopherol alkanoates in which the alkanoate has from one to five carbon atoms, such as acetate, propionate, butyrate or valerate, and alpha-tocopherol acid malonate, succinate and glutarate. Among carotenoids which can be used are ß-carotene, α-carotene, and canthaxanthin.

The relative proportions of the two active agents, vitamin E and carotenoid, in the mixture may vary over a wide range from 1:10 to 10:1 by weight, preferably from 6:4 to 4:6 by weight. Any pharmacologically acceptable carrier or vehicle adapted for oral ingestion by mammals may be employed in the composition such as vegetable oils or foodstuffs. The relative proportions of active agents and vehicle or carrier are not critical but may be adjusted as convenient for administration of a dose of the desired size. Dosages may also vary over a wide range. Doses of the order of 3-4 mg./kg. body weight per day have been found to be effective in the case of a 1:1 by weight mixture of alpha-tocopherol acid succinate and ß-carotene; optimum doses in the case of any particular mixture can readily be determined by conventional procedures.

The following specific example is intended to illustrate more fully the nature of the invention without acting as a limitation upon its scope.

Example

One hundred young male adult randomly bred Syrian hamsters (Mesocricetus auratus) (Lakeview Strain LVG, Charles river Breeding Laboratories) were fed standard Purina Laboratory pellets and water ad libitum. The animals were 60-90 days of age and weighed 95-125g at the beginning of the test procedure. The animals were divided into five groups of 20 animals each and were housed five to a cage and maintained in a controlled environment under standardized conditions of temperature and humidity with an alternating 12 h light-dark cycle. All animals had the right buccal pouches painted three times per week with a 0.5 % solution of 7,12-dimethylbenz(a)anthracene (DMBA) in

heavy mineral oil USP using a number 3 sable brush. Each painting left approximately 0.6 mg. of the DMBA carcinogen on the mucosal surface of the buccal pouch as determined by measurements using the same procedure with (^{14}C) DMBA.

After 14 weeks there were moderate sized tumors in the right pouches of all the animals and carcinogen painting was stopped. The different groups were then treated as follows:

Group 1	no treatment
Group 2	ß-carotene 200µg and alpha-tocopherol acid succinate 200µg in 0.2 ml vegetable oil daily by mouth
Group 3	ß-carotene 400µg in 0.2 ml vegetable oil daily by mouth
Group 4	alpha-tocopherol acid succinate 400 µg in 0.2 ml vegetable oil daily by mouth
Group 5	0.2 ml vegetable oil vehicle only

In each case the material was inserted into the mouth of the animal by syringe. The buccal pouch of the animal was normally closed so that little or no topical application of the material to the tumors occurred. At 22 weeks the tumors in groups 1, 3, 4 and 5 were extremely large. Smaller tumors had coalesced so that one large, extensive mass was observed in all these animals, with diameters measuring from 10-26 mm. The animals in group 2 had one or more small tumors. The animals in groups 1, 3, 4 and 5 at 22 weeks were weak and cachectic and four animals had died during the 21st week. It was decided at that time to sacrifice the animals in groups 1, 3, 4 and 5 in a carbon dioxide chamber for humane reasons and to extend the group 2

animals to 28 weeks. The animals in group 2 were continued with daily feeding of the mixture. There was gradual regression of the tumors until 28 weeks in the group 2 animals so that the tumor burden was lower at that time than that at the start of the treatment at 14 weeks as shown in the drawing. Several of the animals in group 2 at 28 weeks had no grossly visible tumors, although several other animals had tumors larger than those at 14 weeks. The group 2 animals were then sacrificed.

The right buccal pouches of all animals at death were photographed and the tumors measured in each animal. The right pouches were excised and sections of each were fixed in 20% formalin, sectioned in paraffin and stained with hematoxylin and cosin. Cervical lymph nodes were dissected with adjacent tissue and also prepared for histologic study.

Microscopic evaluation demonstrated all tumors to be epidermoid carcinomas. At 22 weeks many of the group 1, 3, 4 and 5 animals had metastatic spread to cervical lymph nodes (about 40%). No tumor was found in cervical lymph nodes in any group 2 animals after 28 weeks.

The tumor burden in cubic millimeters for each group is shown in the plot of the drawing for 14 weeks and 22 weeks, and the burden for the group 2 animals is also shown at 28 weeks.

The results clearly show the remarkable effectiveness of the present invention.

CLAIMS:

1. A composition comprising vitamin E and a carotenoid in proportions from 1:10 to 10:1 by weight together with a pharmacologically acceptable carrier adapted for oral ingestion by mammals.

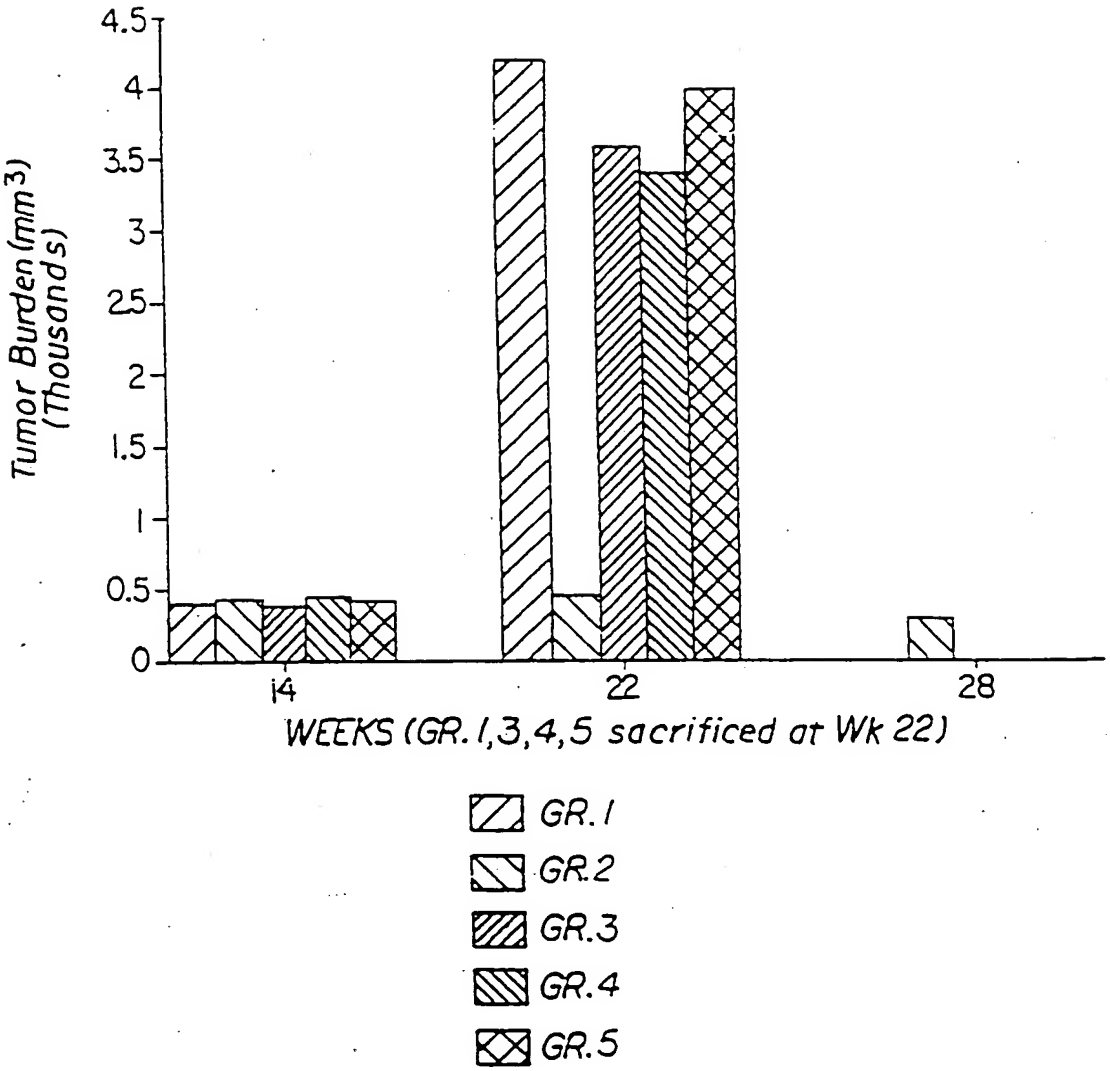
2. A composition as claimed in claim 1 in which said proportions are from 4:6 to 6:4 by weight.

3. A composition as claimed in claim 1 in which said vitamin E is in the form of alpha-tocopherol acid succinate and said carotenoid is ß-carotene.

4. The method of inducing regression of a tumor in vivo in a mammal having said tumor which comprises administering to said mammal by oral ingestion the composition claimed in claim 1.

5. The method of inducing regression of a tumor in vivo in a mammal having said tumor which comprises administering to said mammal by oral ingestion the composition claimed in claim 2.

6. The method of inducing regression of a tumor in vivo in a mammal having said tumor which comprises administering to said mammal by oral ingestion the composition claimed in claim 3.


Tumor Burden (mm3) (Thousands)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
14
22
28
WEEKS (GR. 1,3,4,5 sacrificed at Wk 22)
GR. 1
GR. 2
GR. 3
GR. 4
GR. 5

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US90/01259

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

INT CL[5] A61K 31/07, 31/355; C07C 35/18, C07D 311/04

U.S. CL.: 514/458, 725, 549/408, 568/824

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
US	514/458, 725, 568/824, 549/408

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No [13]
Y	Chemical Abstracts, volume 109, no. 17, issued 1988, October 24 (Columbus, Ohio, U.S.A.) D. Deng et al., "Effect of -carotene on sister chromatid exchanges induced by MNNG and aflatoxin B_1 in V79 cells", see page 29, column 2, abstract No. 142177f, Zhonghua Zhongliu Zazhi, 1988, 10(2) 89-91 (Ch)".	1-6
Y	Chemical Abstracts, volume 99, no. 9, issued 1983, August 29 (Columbus, Ohio, U.S.A.) V.N. Rumbesht et al; "Effect of a lipid-soluble vitamin complex on development of induced tumors and certain indices of the body's immune reactivity", abstract no. 69289n, Eksp. Onkol. 1983, 5(3), 42-45 (Russ)".	1-6

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
18 APRIL 1990	11 JUN 1990
International Searching Authority	**Signature of Authorized Officer**
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